HIV Brain Latency as Measured by CSF BcL11b is Linked to Disrupted Brain Cellular Energy in virally suppressed HIV infection

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Background

- Chronic brain injury slowly worsens in some HIV patients:
  - even in virally suppressed
  - without confounds
  - without brain inflammation (by MRS criteria) (Cysique et al. 2018)
- The cause is unclear:
  - ? Related to brain HIV latency (CSF BcL11b) (Desplats 2013) **The primary hypothesis**
  - ? Related to low level inflammation in CSF (neopterin)
  - ?low level restricted brain infection (CSF tat)

Lucette A. Cysique, Lauriane Jugé, Thomas Gates, Michael Tobia, Kirsten Moffat, Bruce J. Brew, Caroline Rae. Covertly active and progressing neurochemical abnormalities in suppressed HIV infection. Neurology - Neuroimmunology Neuroinflammation 2018.
Desplats P, Dumaop W, Smith D, Adame A, Everall I, Letendre S et al. Molecular and pathologic insights from latent HIV-1 infection in the human brain. Neurology 2013.

AIMS

1. Determine relationship between MRS metabolites (baseline and at 18months) CSF BcL11b, neopterin, NFL and tat.

2. Determine relationship between the latter indices and baseline HAND status and neurocognitive decline at 18 months.

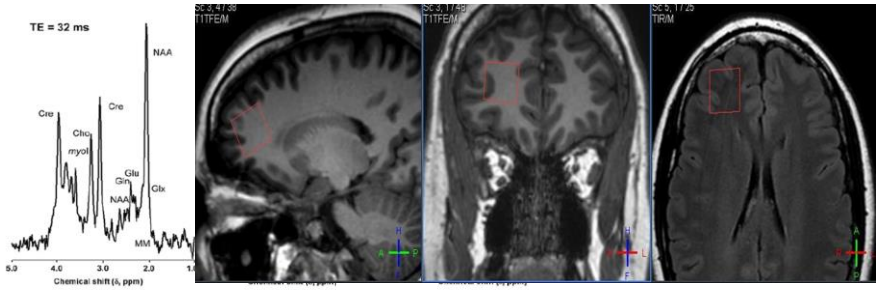
Table 1: demographic, clinical and laboratory characteristics in the study sample

N	26
Age (mean (SD))	57.31 (7.05)
Education (mean (SD))	13.81 (2.88)
Sex (% male)	100
<200 Nadir CD4 % (%)	65.4
Median Current CD4 (cells/mL)	572
Median HIV duration (years)	19.5
Historical AIDS (%)	76.9
Treated during early HIV infection (%)	26.9
Plasma HIV RNA %undetectable	92
CSF HIV RNA %undetectable	96
Median Current CART duration (months)	24
Current HAND (%)	61.5
ANI / MND, HAD (N,%)	11, 42/ 4, 15.4 / 3, 11.5
History of HAND (%)	23.1
Depressive symptoms (%depressed)	15
CSF BcL11b expression	0.42 (0.16)
CSF neopterin (mmol/L)	14.32 (5.16)
Log_{10} CSF NFL (pg/mL)	2.94 (0.17)
CSF tat (pg/ML)[1] (% detectable)	19

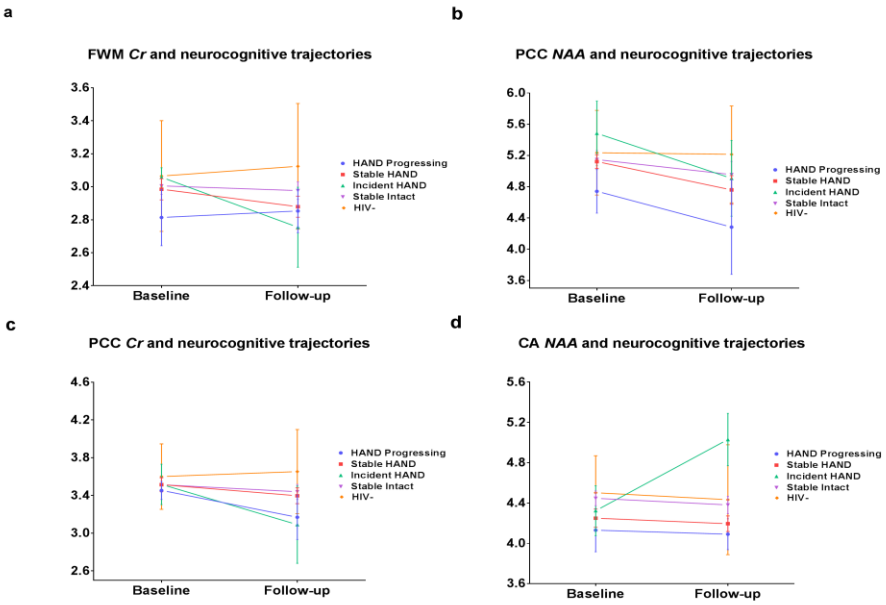
[1] *Ranges from 27.52-375.15*

Data collected at baseline & 18-month

- Standard neuropsychological test battery covering 7 domains
- ^{1}H MRS on Philips 3T Achieva Quasar Dual imaging system using point-resolved spectroscopy (PRESS) in Frontal White Matter (FWM), Posterior Cingulate Cortex (PCC) and Caudate area (Caudate) to measure:



MRS and neurocognitive trajectories



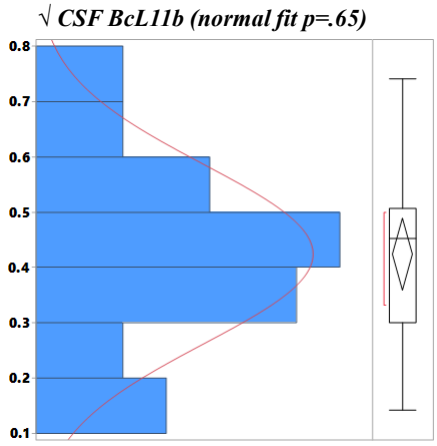
CSF biomarker quantification methods

- ***CSF neopterin:*** high-performance liquid chromatography (HPLC) with a lower limit of detection of 8 nmol/L.
- ***CSF NFL:*** enzyme linked immunosorbent assay (ELISA) kit (Uman Diagnostics AB, 10-7001).
- ***CSF tat:*** sandwich enzyme-linked immunosorbent assay by Nath et al. NIH laboratory *(Johnson et al 2016)*.
- ***CSF BcL11B:*** Western blots resolved on 4-12% Bis-Tris gels (Novex by Life Technologies) & transferred onto a filter membrane (Invitrolon PVDF, 0.45m pores, Life Technologies); Membrane stained with Bcl11b antibody (1:1000, Bethyl laboratories) overnight, followed by secondary staining with goat anti-rat IgG (800CW IRDye, 1:10,000) for 2 hours. Jurkat cell lysate was used as a positive control, the healthy samples as a negative control and β-actin as a loading control (1:1000, Sigma-Aldrich A5441).

**Johnson TP, Nath A. Protocol for Detection of HIV-Tat Protein in Cerebrospinal Fluid by a Sandwich Enzyme-Linked Immunosorbent Assay. Methods Mol Biol 2016;1354:343-352.*

CSF biomarkers collected at baseline *only*

- ***CSF BcL11b*** expression was square root (√) transformed.
- ***CSF neopterin*** was not skewed and analysed as is.
- ***CSF NFL*** was $\log_{10}$ transformed.
- ***CSF tat*** was highly skewed and was analysed as a binary variable: detectable=5/undetectable=21



Cross-sectional statistical rationale

- Test of Associations between the baseline CSF biomarkers: CSF BCL11b was *not* significantly correlated with either CSF neopterin (moderate effect size), CSF NFL (small effect size) and CSF Tat (small effect size).
- Test of univariate associations between CSF biomarkers and brain metabolites: **higher CSF BCL11b expression was associated with lower with FWM Cr (-.37).**
- **Test of our primary hypothesis**: the subsequent analyses focused on the effect of CSF BCL11b on the FWM Cr.

The statistical rationale aims at reducing the total number of comparisons while addressing our hypothesis

	trivial	small	moderate	large	very large	nearly perfect	perfect
Correlation	0.0	0.1	0.3	0.5	0.7	0.9	1
Diff. in means	0.0	0.2	0.6	1.2	2.0	4.0	infinite
Freq. diff.	0	10	30	50	70	90	100
Rel. risk	1.0	1.2	1.9	3.0	5.7	19	infinite
Odds ratio	1.0	1.5	3.5	9.0	32	360	infinite

Cross-sectional Statistical analyses

- Regression models, BcL11b as primary predictor
  - Adjusted separately for CSF neopterin, NFL or Tat
  - Also run with relevant demographic* and HIV biomarkers covariates*
  - Outcome: FWM Cr.

*Age, education, having a history of HAND, <200 Nadir CD4 (yes/no); current CD4+ T cell count (cell/mL); history of AIDS (yes/no), current cART duration (months); Treated during early HIV infection (yes/no), HIV duration (years), depressive symptoms (yes/no)

Longitudinal statistical rationale

- Is ***BCL11b*** predictive of any brain metabolite change at 18-month (FWM, PCC, Caudate) in an unadjusted model
- Is ***BCL11b*** predictive of any brain metabolite change[1] at 18-month (FWM, PCC, Caudate) in 3 separate regression model adjusting for neopterin, NFL and tat?
- Each model included a time effect, and a time*BCL11b non-linear effect.
- Also run with relevant demographic* and HIV biomarkers covariates*.

[1]As for the larger cohort, there are changes in brain metabolites that depends on the neurocognitive trajectories (Cysique et al. 2018 N2)

*Age, education, having a history of HAND, <200 Nadir CD4 (yes/no); current CD4+ T cell count (cell/mL); history of AIDS (yes/no), current cART duration (months); Treated during early HIV infection (yes/no), HIV duration (years), depressive symptoms (yes/no)

Results

Correlations: r or ρ and (p-value) between the CSF biomarkers

	√ CSF BCL11b	CSF neopterin	Log_{10} CSF NFL	CSF tat [1] (detectable/ undetectable)
√ CSF BCL11b		-.35 (.08)	.22 (.27)	-.17 (.39)
CSF neopterin (mmol/L)	-.35		-.02 (.98)	.30 (.14)
Log_{10} CSF NFL (pg/mL)	.22	-.02		.08 (.68)
CSF tat [1] (detectable/ undetectable)	-.17	.32	.08	

[1] non-parametric statistics were conducted for tat

None of the correlations were significant at p<.05

P-value based on correlation statistics between the CSF biomarkers, demographic and clinical characteristics

	√ CSF BCL11b p	CSF neopterin p	Log_{10} CSF NFL p	CSF tat [1] p (detectable / undetectable)
Age (years)	.30	.19	**.03**	**.04**
Education (years)	.83	.67	.93	.25
<200 Nadir CD4 %	.90	.95	.33	.78
Current CD4 (cells/mL)	.61	.82	.94	.24
HIV duration (years)	.17	.56	**.13**	.30
Historical AIDS (yes/no)	.36	.84	.59	.34
CART duration (months)	.67	.51	.88	**.10**
Treated during early HIV infection (yes/no)	.31	.99	.70	.48
History of HAND % (yes/no)	.90	.92	**.02**	.85
Depressive symptoms (yes/no)	.22	.18	.53	.17

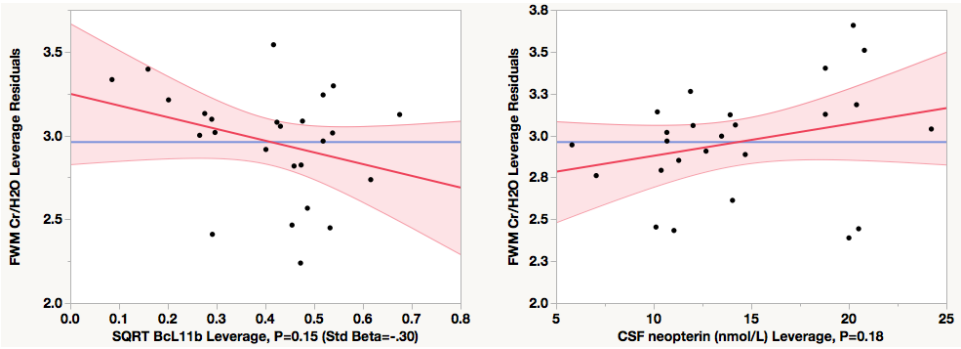
[1] non-parametric statistics were conducted for tat

Because of the relatively small sample size, correlation with p-value <.15 were considered in the final multivariate models

Correlations: r and ρ and (p-value) between the CSF biomarkers and brain metabolites				
	√ CSF BCL11b	CSF neopterin	Log_{10} CSF NFL	CSF tat [1] (detectable/ undetectable)
FWM NAA	-.18 (.39)	.02 (,93)	.13 (.54)	-.29 (.15)
FWM Cr	**-.37 (.06)**	**.38 (.06)**	**.02 (.93)**	**-.25 (.21)**
FWM Cho	.24 (.24)	-.32 (.11)	.11 (.60)	-.50 (.009)
FWM MI	-.05 (.81)	.29 (.15)	.29 (.15)	.11 (.59)
FWM Glx	.19 (.36)	-.36 (.08)	.10 (.62_	-.06 (.77)
PCC NAA	-.11 (.58)	.22 (.29)	-.17 (.41)	-.07 (.72)
PCC Cr	-.18 (.38)	.28 (.18)	-.31 (.12)	0 (1.0)
PCC Cho	-.16 (.43)	.29 (.15)	-.37 (.06)	-.03 (.87)
PCC MI	-.21 (.29)	.38 (.06)	-.31 (.13)	-.08 (.70)
PCC Glx	.03 (.87)	.01 (.98)	-.36 (.07)	.01 (.97)
Caud NAA	-.17 (.41)	.21 (.31)	.02 (.91)	.10 (.63)
Caud Cr	.06 (.79)	.14 (.50)	-.23 (.27)	.03 (.89)
Caud Cho	.04 (.86)	-.08 (.71)	.01 (.96)	.03 (.87)
Caud MI	-.27 (.19)	.24 (.27)	.02 (.91)	.08 (.69)

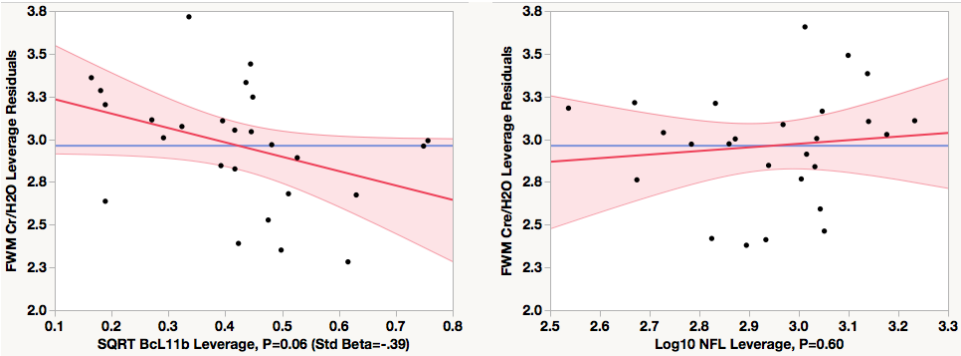
Cross-sectional Results

A higher CSF BcL11b is moderately to largely associated with lower FWM Cr even when adjusted for CSF neopterin



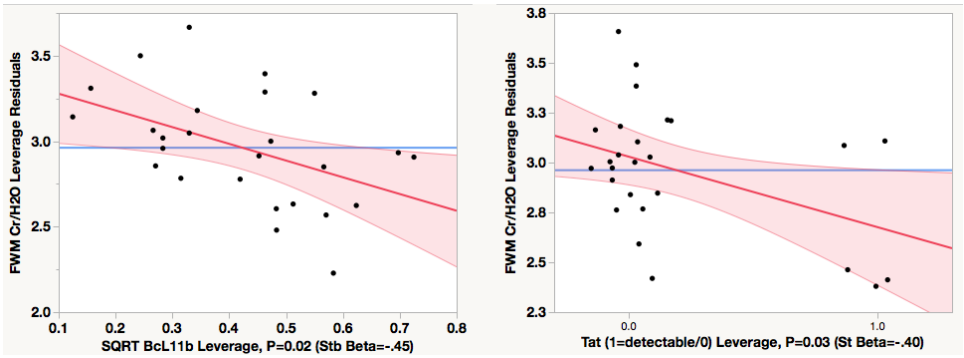
An effect leverage plot, also known as added variable plot or partial regression leverage plot, shows the unique effect of a term in the model

A higher CSF BcL11b is moderately associated with lower FWM Cr while adjusting for CSF NFL



An effect leverage plot, also known as added variable plot or partial regression leverage plot, shows the unique effect of a term in the model

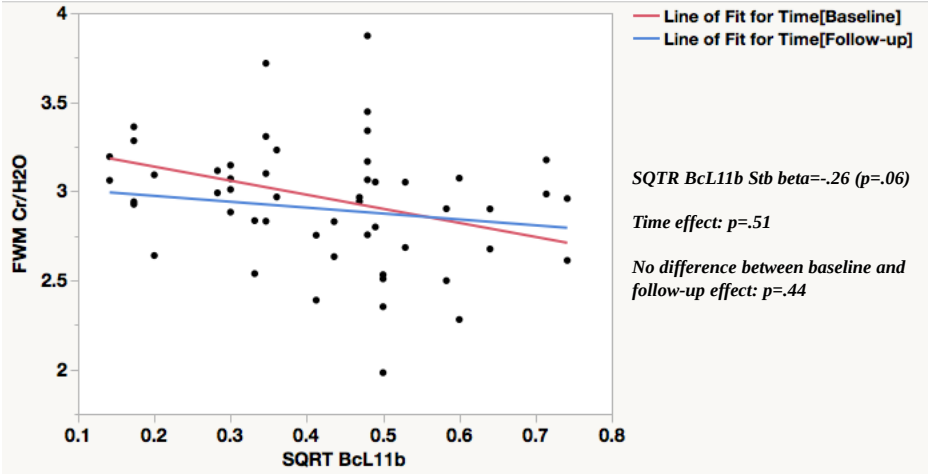
A higher CSF BcL11b is moderately to largely associated with lower FWM Cr even when adjusted for CSF tat



An effect leverage plot, also known as added variable plot or partial regression leverage plot, shows the unique effect of a term in the model

Longitudinal Results

A higher CSF BcL11b is moderately associated with lower FWM Cr at baseline and follow-up



Other results

- Adjusted model for neopterin, NFL and tat showed no time effect.
- There was a similar magnitude of the BcL11b association to lower FWM Cr in the longitudinal models to that of the cross-sectional models.
- In both cross-sectional and longitudinal analyses, there was no association of the BcL11b effect on lower FWM Cr to the baseline HAND status or that of the neurocognitive decline.

Conclusions

- There was a specific association between BcL11b and Cr in the FWM which was not detected in any other tested brain area
- The magnitude of BcL11b association to lower FWM Cr was consistent despite other CSF biomarkers adjustments and confirmed in longitudinal and the cross-sectional analyses.
- The lack of relation to baseline HAND status or to neurocognitive decline suggests a mostly subclinical effect
- This is an hypothesis-generating finding that needs to be replicated in a larger sample.
- Targeted ^{1}H MRS may offer a non-invasive option to measure HIV brain latency.

Acknowledgements

- Thanks to our research participants without whom this research would not be possible.
- This work was supported by the Peter Duncan Applied Neuroscience Unit at the St. Vincent's Hospital Applied Medical Research Centre, Sydney Australia (Head of Group. Prof. Bruce Brew)
- And NHMRC CDF APP1045400 (Cysique).
- Thanks to our collaborators at NIH & JHU Drs. Nath and & Johnson.